IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 4, Issue 3, Mar 2016, 51-56

ELISA AND ICT TECHNIQUES IN THE DETECTION OF ANTI- RUBELLA VIRUS ANTIBODIES IN ABORTED WOMEN IN AL -NASIRIYAH CITY, THI-QAR, IRAQ

INTIDHAAR N. ABID & AHMED N. FAYAD

Department of Pathological Analysis, College of Science, University of Thi-Qar, Thi-Qar Province, Iraq

ABSTRACT

A total of 200 serum samples were collected from Bent Al-Huda hospital in Al -Nasiriyah city, Thi- Qar province, Iraq. These samples were collected from aborted women in different ages and cases to detect of rubella virus by Enzyme Linked Immuno Sorbent Assay (ELISA) and Immuno chromatography (ICT) techniques.

It was founded from the recent study 162 (81%) of serum samples were positive by IgG ELISA, and 105 (52.5%) were positive by IgG with ICT technique. All serum samples were registered negative results to IgM anti- rubella virus by two techniques.

The results of ELISA technique were appeared the higher rate (97.95%) for anti-rubella virus antibodies was observed in second age group ((21-30)years) and a no significant differences were observed in the seropositivity concerning to age groups(P value:> 0.05).

In ICT, the higher rate (85.71%) of anti-rubella virus antibodies was observed in the second group of ages, with the presence of significant among the age groups(P value:< 0.05). According to the cases, the higher rate (96%) of anti-rubella virus antibodies was in bleeding in early pregnancy case which observed with ELISA technique, and no founded significant differences among cases groups P value:> 0.05), while 90.9% of seropositivity fetal death cases was resulted with ICT technique, and were founded highly significant differences among the rates of seropositivity against rubella virus(P value:< 0.01).

The sensitivity and specificity were registered 95% and 61% respectively with ELISA test, while the lower sensitivity and sensitivity values were with ICT test, they were 85% and 57% respectively, and the statistical analysis was appeared the existence of significant differences (P value:< 0.05) between the two tests.

KEYWORDS: Rubella, Elisa, ICT

INTRODUCTION

Rubella is an infection caused by a rubella virus [1]. Rubella virus is the sole member of the genus Rubivirus in the Togaviridae family, only one serotype has been identified [2].Rubella virus is a spherical (40-80) nm, positive-sense, single-stranded RNA virus with spike-like haemagglutinin-containing surface projections. An electron-dense (30-35) nm core is surrounded by a lipoprotein envelope [2, 3].The name Rubella comes from the Latin word meaning “little red”. In 1814, it was first discovered to be a separate disease from measles in German medical literature thus receiving its nickname the “German measles”, In 1914, Hess postulated a viral etiology and in 1914 Norman Gregg reported congenital

cataracts in 78 infants whose mothers had maternal rubella in early pregnancy [4].

Rubella virus is transmitted (congenital) to a fetus by an infected mother [5] Acquired Rubella (non congenital) is transmitted via airborne droplet emission from the upper respiratory tract of active cases; can be passed along by the breath of people sick from rubella; also can be spread by coughing or sneezing .The virus may also be present on the skin, there is no carrier state, the reservoir exists entirely in active human cases. The disease has an incubation period of two to three weeks [6, 7]. Rubella generally is a mild rush fever disease when acquired in childhood, but when infection occurs during the first months of gestation leading to fetal death, miscarriage, stillbirth or infants with a pattern of birth defects, known as congenital rubella syndrome CRS [8].Rubella has symptoms that are similar to those flu. , however, the primary symptom of rubella virus infection is the appearance of rash (exanthema) on the face which spreads to the trunk and usually fades after three days. Other symptoms include low-grade fever, swollen glands(sub occipital & posterior cervical lymphadenopathy),joint pains , headache and conjunctivitis .The virus has a tetatogenic properties and is capable of crossing placenta and infecting the fetus [9].20-50% of infected persons are asymptomatic, the laboratory diagnosis of rubella is required, since clinical diagnosis is often inaccurate [10].Viral isolation may occasionally be warranted particularly during infections in pregnancy[11], and as a result of the non-cytopathic effect of rubella virus in cell cultures, it is not usually recommended[12].Nucleic acid amplification techniques have been developed since the 1990 for the detection of rubella virus RNA in clinical samples of orophangeal sources in serum or saliva[12,13]. Diagnosing rubella relies on the same twin techniques that using in measles. Because it mimics other diseases ,rubella should not be diagnosed on clinical grounds alone. IgM antibodies to rubella virus can be detected early using ELISA technique, other conditions and infections can lead to false positives. However, and the IgM test should be augment by an acute and convalescent measurement of IgG antibody. It is important to know whether the infection is indeed rubella, especially in women because if so, they will be immune to reinfection [14]. In recent years, other assays such as Immunochromatography have been developed for rubella testing [15].

MATERIALS AND METHODS

In this study the 200 blood samples were collected from aborted women after taking written informed consent in Bent-Al Huda hospital in to 10 ml of blood sample was placed in sterile tubes without anticoagulant and were stored overnight at room temperature (18-25) C° for serum separation. The samples were then centrifuged at high speed 3000 rpm for 5 minutes. The serum was transferred into a single clean, sterile cryovial to conserve at -20C° until assayed [16].

Rubella antibodies were detected by the enzyme linked immune sorbent assay (ELISA) IgG and IgM Rubella kit (Foresight, Acon Laboratories, San Diego, USA) and specific colloidal gold solid-phase Immunochromatography (ICT) Rubella IgM and IgG kit (Weifang Kanghua, Biotech, Shandong, China).

RESULTS

From the 200 total serum samples examined by ELISA and ICT for the presence of antibodies of Rubella virus, 162(81%) were positive by IgG ELISA. A 105(52.5%) were positive by IgG ICT. All the tested samples showed negative results to IgM antibodies by two techniques.

The highest positive age group was observed in 21-30 years group with 48(97.95%) and 42(85.71%) in ELISA and ICT respectively. Statistical analysis by using chi-square was appeared no significant among age groups with anti-

rubella virus antibodies by ELISA technique (P value:> 0.05). Dependent on ICT technique, it was presence of significant differences in the rate of seropositivity among age groups (P value:< 0.05) (table 1).

Table 1: The Seropositivity against Rubella Virus Antibodies According to Age Groups of Women

Age Groups		ELISA Positive(+)		ICT Positive(+)	
Age(Years)	Number	Number	Percentage	Number	Percentage
14-20	49	40	(81.6%)	24	(48.97%)
21-30	49	48	(97.95%)	42	(85.71%)
31-40	50	39	(78 %)	29	(58%)
41-45	52	35	(67.3%)	10	(19.23%)
The Total	**200**	**162**	**(81%)**	**105**	**(52.5%)**
P Value		**> 0.05**		**< 0.05**	

Table 2 displayed the Seropositivity according to case group, the bleeding in early pregnancy was registered higher percentage (96%) for seropositivity among other cases in ELISA, while the fetal death case was registered (90.9%) in ICT technique.

The anti-rubella virus antibodies based ELISA positive results were not statistically significant (P value:> 0.05), but ICT was highly significant different among the rates of seropositivity against rubella virus(P value:< 0.01).

Table 2: The Seropositivity against Rubella Virus Antibodies According to Cases Groups

Types of Cases		ELISA Positive(+)		ICT Positive(+)	
Case	Number	Number	Percentage	Number	Percentage
Recurrent abortion	60	55	(91.6%)	51	(85%)
Congenital anomalies	68	40	(58.8%)	16	(23.52%)
Bleeding in early pregnancy	50	48	(96%)	18	(36 %)
Fetal death	22	19	(86.36%)	20	(90.9%)
The Total	**200**	**162**	**(81%)**	**105**	**(52.5%)**
P Value		**> 0.05**		**< 0.01**	

The results in Table 3 show the sensitivity and specificity of ELISA and ICT techniques. It was appeared high sensitivity (95%) in ELISA technique, and in specificity was (85%), while ICT technique was registered (61%) and (57%) in sensitivity and specificity respectively. The differences between both techniques were found to be significant (P value :< 0.05).

Table 3: The Sensitivity and Specificity of ELISA and ICT

	ELISA	ICT
Sensitivity	95%	61%
Specificity	85%	57%
P Value	**< 0.05**	

DISCUSSIONS

The seriousness of Rubella was not appreciated until 1941, when the association was made between certain sever birth defects and maternal infection during the first trimester (3 months) of pregnancy, a condition called congenital rubella syndrome. If a pregnant women contracts the disease during this time, there is about a 35% incidence of serious damage

(17).

The detection of specific rubella antibodies has been of great importance due to reduce risk of the virus, serological tests are used recently to determine immune status and acute rubella infection (18).

Our results appeared the presence of anti-rubella virus IgG antibodies were in high rates in serum samples that tested by ELISA and ICT. But all samples showed negative for IgM by two techniques. These results may be due to old infections of rubella.

The presences of IgM antibody indicates an acute infection, and is usually undetectable after 2 months. Rubella IgG will appear almost as early as the IgM, but then persists for a life time (19), these results are in agreement with those obtained by Sangeetha *et al.,* (2012) who found 80.75% and 46.58% of serum samples were positive by IgG ELISA and IgG ICT respectively, and all samples were negative for IgM antibodies by both techniques (15).

This study was showed the highest positive age group to anti rubella virus antibodies was observed in 21-30 years group, this was probably result from non immune to rubella in women of this age group.

In relation to seropositivity according to case group, the recent study was showed, the higher numbers for anti rubella virus were founded in bleeding in early pregnancy and fetal death cases in ELISA and ICT respectively.

Many mothers who contract rubella within the first critical trimester either have a miscarriage or a still born baby (20), miscarriage is the most common causes of bleeding in early pregnancy (21).

Sensitivity and specificity are gauges used to evaluate a clinical test (22). The recent study compared the sensitivity and specificity between the two techniques. ELISA technique showed a high percentage of sensitivity (95%) and specificity (85%), these results were similar to results of Field *et al.,* (1988) who showed a sensitivity of 97% and specificity of 95.6% (23). Our results showed the values of sensitivity and specificity with ICT was lower when compared with ELISA test, as the percentage 61% and 57% respectively. These results were disagreed with those obtained by Mu Ying et al., (2010) who found 100% sensitivity and specificity with ICT (24). Our results are agreement with Terada et al., (2002) they compared ELISA with ICT technique for detection of IgG antibodies against Rubella, in their study ELISA showed 100% in sensitivity and specificity, ICT showed 99.35% of sensitivity and 100% of specificity (25).

CONCLUSIONS

In conclusion, a high rates of aborted women have antibodies against rubella virus, and our results conclude that, the ELISA technique is standard technique and have high sensitivity and specificity for detection of anti-rubella antibodies. ICT technique more rapid, yield titer in short time but less sensitivity and specificity compared with ELISA technique

The study recommend screening women of childbearing age for antibodies against rubella virus, and use ICT in screening of rubella virus but not use it in confirm diagnosis of infection, as well as to use ELISA technique in confirm the infection with rubella virus.

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